Chemical and Enzymatic Oxidation of 2-Aryl-1,3-oxathiolanes: Mechanism of the Hepatic Flavin-Containing Monooxygenase

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Abstract: The reaction of $NaIO_4$, H_2O_2, and highly purified and microsomal hog and rat liver flavin-containing monooxygenase with 2-aryl-1,3-oxathiolanes was investigated. The ρ values determined from Hammett plots for the rate of S-oxygenation are consistent with substantial nucleophilic character for the chemical reaction but this does not preclude radical character in the reaction. For the biotransformation reactions, the data provide evidence for a minor role of cytochrome P-450 in the S-oxygenation of 2-aryl-1,3-oxathiolanes, but the flavin-containing monooxygenase represents by far the major pathway for *S*-oxide formation. The diastereochemical outcome of the S-oxygenation of 2-aryl-1,3-oxathiolanes was determined and, in general, hog liver flavin-containing monooxygenase demonstrated considerable S-oxygenation stereoselectivity while rat liver flavin-containing monooxygenase (FMO) was markedly less stereoselective. The presence of the minor cis S-oxide diastereomer in each case is due to incomplete diastereomeric processing by each enzyme (FMO and cytochrome P-450) and not to a competing, achiral nonenzymatic process. 2-Aryl-1,3-oxathiolane *S*-oxides are also oxygenated a second time by H_2O_2 or hog or rat liver microsomal and highly purified FMO. The immediate S,S-dioxygenated product is not stable and is rapidly converted to the corresponding benzaldehyde. That the chemical and enzymatic oxygenation of 2-aryl-1,3-oxathiolane *S*-oxides is much slower than its corresponding sulfide is quite apparent from the large dependence on the nature of the para substituent. The reactions of 2-aryl-1,3-oxathiolanes with H_2O_2 and FMO serve to demonstrate the electronic and stereochemical requirements for S-oxygenation of dialkyl sulfides and provide evidence that rat and hog liver FMO are two different forms of the same enzyme.

The flavin-containing monooxygenase (FMO) (E.C. 1.14.13.8, *N,N*-dimethylaniline monooxygenase) catalyzes the NADPH- and oxygen-dependent oxidation of a wide variety of tertiary amines and nucleophilic sulfur-containing drugs, pesticides, and xenobiotics.[1,2] The mechanism of FMO is known in some detail for hog liver[3-6] and it is reasonable to assume that the enzymatic steps will be similar in other forms of mammalian FMO. Studies to date indicate that the resting form of the enzyme is the long-lived, stable 4a-hydroperoxyflavoprotein intermediate.[3,4] Oxygenatable substrate is not essential for 4a-hydroperoxyflavin formation of FMO and there is no evidence to suggest that substrate requires activation by the enzyme.[4-7] The 4a-hydroperoxyflavin intermediate is formed in a fast step upon the reaction of molecular oxygen and reduced flavin.[3,6] There is no evidence for enzymic intermediates in the transfer of oxygen to nucleophilic substrates and model studies employing N^5-ethyl-N^{10}-alkyl-4a-hydroperoxyisoalloxazines have been successfully used to investigate the nature of FMO oxygenation.[8-12] The rate-limiting enzymic step for hog liver FMO is postulated to be decomposition of the flavin pseudobase or release of $NADP^+$ from the enzyme.[6,7] Other enzymatic steps may also be partially rate limiting for FMO from other species because the V_{max} of hog liver FMO is increased several fold in the presence of lipophilic primary amines like *n*-octylamine, while stimulation of rat liver FMO by *n*-octylamine is variable.[13-15] If the rate of dehydration of the flavin-pseudobase intermediate of the FMO reaction is accelerated by either an increase in pH or the addition of *n*-octylamine, it is possible that transfer of oxygen to the substrate may become the rate-determining step of the reaction. For a peroxide–nucleophile reaction, the distinction of single-electron transfer and S_N2 mechanisms using substituent effects of the substrate alone are unlikely to yield a clear answer.[16] However, substituent effects coupled with stereochemical product analysis of the oxygenation of nucleophilic sulfides could potentially distinguish these mechanisms. A large number of kinetic studies demonstrate that any compound that can be oxidized by an organic hydroperoxide can serve as a substrate for FMO.[17-19] The studies performed to date do not suggest a role of free-radical processes and have been best explained as occurring through nucleophilic attack of substrate upon the terminal oxygen of the 4a-hydroperoxyflavin although a radical mechanism cannot be excluded.[19] In general, electron-rich sulfur-containing compounds are better substrates for FMO than electron-deficient sulfur-containing compounds,[20-22] but a complete examination of this question has not been performed. FMO has been shown to stereoselectively S-oxygenate a number of compounds,[23,24] but an exhaustive study is lacking.

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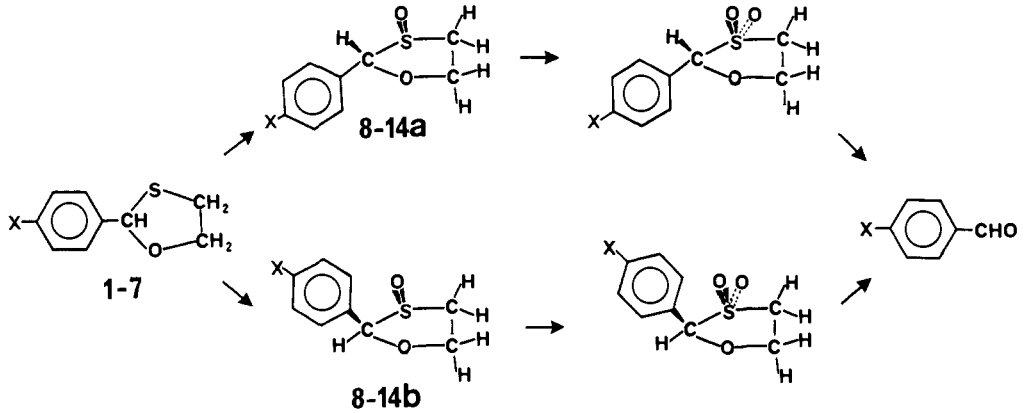
Scheme I. Overall Oxidative (Bio)chemical Transformation of 2-Aryl-1,3-oxathiolanes to Their Corresponding Benzaldehydes



1, X = HO- 8, X = HO-
2, X = CH_3O- 9, X = CH_3O-
3, X = CH_3- 10, X = CH_3-
4, X = H- 11, X = H-
5, X = Cl- 12, X = Cl-
6, X = CN- 13, X = CN-
7, X = NO_2- 14, X = NO_2-

Recent reports demonstrate that different tissues contain different forms of FMO.[25-27] Thus, reports from a number of laboratories suggest that FMO present in rabbit liver and lung are immunologically distinct proteins. Variations in the liver FMO enzyme from different species are less pronounced,[28] but a recent report demonstrates that hog liver microsomal FMO possesses tertiary amine N-oxygenation stereoselectivity opposite to that of rat liver FMO, suggesting the presence of different forms of the same enzyme.[29] Little is known concerning the way different forms of FMO S-oxygenate dialkyl sulfides, but the biochemical basis for the oxidation of sulfides to sulfoxides has been studied and FMO is the principal enzyme involved in the oxygenation of lipophilic thioethers.[1,2,22]

Recently, a number of reports have described the fungal biotransformation of 1,3-oxathiolanes[30] and the corresponding dithiolanes,[31,32] but no studies of mammalian S-oxygenation of these classes of sulfides have been reported. In fungi, sulfoxidation occurs to give a single major diastereomeric product and the relative stereochemistry has been assigned to the trans configuration with zero or low enantiomeric enrichment.[30] Other studies have demonstrated a stereopreference for the *pro-R* sulfur atom during microbial oxidation of dithiolanes to the *trans*-1,3-dithiolane *S*-oxide.[32]

In an attempt to examine the electronic and stereochemical requirements for the S-oxygenation of dialkylsulfides by FMO, we have prepared 2-aryl-1,3-oxathiolanes and investigated the oxidation of 2-aryl-1,3-oxathiolanes and their *S*-oxides with hydrogen peroxide, sodium periodate, and microsomal and highly purified hepatic FMO from hog and rat. 2-Aryl-1,3-oxathiolanes were chosen as a model substrate since (a) dialkyl sulfides are less reactive to oxidation than some other nucleophilic sulfur substrates, (b) S-oxidation of 2-aryl-1,3-oxathiolanes produces two diastereomers stable to pyramidal inversion, and (c) S-oxidation of 2-aryl-1,3-oxathiolane *S*-oxides only very slowly produces highly reactive *S,S*-dioxides which very efficiently decompose to the readily identifiable benzaldehydes (Scheme I). The latter two properties of 2-aryl-1,3-oxathiolanes and their *S*-oxides greatly simplify the kinetic analysis for S-oxygenation. From the results of these studies, we conclude that hog liver FMO is highly stereoselective while rat liver FMO is less so and that the oxidation of sulfides by FMO involves a significant amount of nucleophilic character.

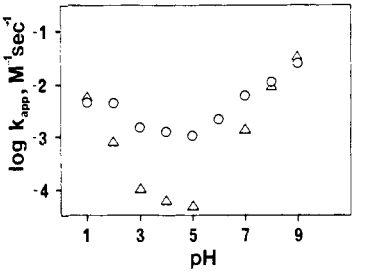


Figure 1. Plot of the apparent second-order rate constants versus pH for the H_2O_2-catalyzed oxidation of 2-(4-nitrophenyl)-1,3-oxathiolane (○) and *trans*-2-(4-nitrophenyl)-1,3-oxathiolane *S*-oxide, compound **14a** (△).

Results

Reaction of 2-(4-Nitrophenyl)-1,3-oxathiolane with Hydrogen Peroxide or Sodium Periodate. At pH 7.0, the reaction of 2-(4-nitrophenyl)-1,3-oxathiolane, **7**, with excess hydrogen peroxide or sodium periodate was observed to follow pseudo-first-order kinetics for 4–5 half-lives. A plot of the logarithm of the product formed vs time gave a straight line and an excellent correlation coefficient, *r*, of 0.99. The corresponding 2-(4-nitrophenyl)-1,3-oxathiolane *S*-oxide, **14a,b**, was the only detectable product, although, at extremely long reaction times, it too was oxidized and decomposed to 4-nitrobenzaldehyde. Of the two diastereomers that could form, a major trans diastereomer (**14a**) was formed in greater than 85% yield. The second-order rate constants for oxidation were determined from the slope of a plot of k_{obs} vs hydrogen peroxide or sodium periodate concentration. Such plots had zero intercepts and had an excellent correlation coefficient, *r*, of 0.99 and were linear throughout the range of hydrogen peroxide or sodium periodate concentration from 0 to 140 mM. The second-order rate constants that were determined from plots of k_{obs} vs hydrogen peroxide or sodium periodate in two separate experiments gave rate constants that only varied between 3 and

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Table I. Microsomal S-Oxygenation of 2-Aryl-1,3-oxathiolanes

2-aryl-1,3-oxathiolane		hog liver microsomes, nmol min^{-1} (mg of protein)$^{-1}$			rat liver microsomes, nmol min^{-1} (mg of protein)$^{-1}$		
compd	aryl	comp[a]	−NOA[b]	+heat inactiv[c]	comp[a]	−NOA[b]	+heat inactiv[c]
2	4-CH_3O	7.65			3.52		
3	4-CH_3	3.85	0.57	ND[d]	4.75	0.51	0.03
4	4-H	3.53	1.03	0.79	1.57	0.85	0.42
5	4-Cl	3.32	1.08	ND[d]	2.27	0.20	0.15
7	4-NO_2	6.80	1.48	ND[d]	2.85	0.50	0.01

[a] The complete system contained 50 mM phosphate buffer, pH 7.8, the NADPH generating system, 200 mM substrate, 4 mM *n*-octylamine, and 0.56–0.87 mg of microsomal protein incubated for 3 min at 33 °C as described in the Experimental Section. Data represent the average of four to eight determinations. [b] Complete system without *n*-octylamine (NOA). [c] Complete system without *n*-octylamine pretreated with heat inactivation as described in the Experimental Section. Heat-inactivated microsomes used in this study retained 85–100% of their cytochrome P-450 activity (ref 29). [d] Not detectable.

4%. To investigate the effect of pH on the reaction of **7** and hydrogen peroxide, we first established the stability of **7** in the range from pH 1 to 10. Compound **7** was stable between pH values of 2 and 9. At pH 1, the half-life for hydrolysis of compound **7** was 96 h, and at pH 10, the half-life for hydrolysis was 195 h. The use of various concentrations of HCl (pH 1.3), acetate (pH 5.0), and phosphate (pH 7.2) buffers (i.e., 0.08–0.4 M) showed that no buffer catalysis was occurring for the H_2O_2-catalyzed oxidation of **7**. The results of these studies are similar to other results which demonstrate that general-acid or general-base catalysis does not occur for the ROOH-mediated oxidation of sulfides.[8,16,17]

The second-order rate constant for the reaction of **7** and H_2O_2 was essentially constant over the range of pH 3–6 (Figure 1). However, at acidic (pH 1–2) and basic (pH 7–9) pH values, significant increases in H_2O_2-catalyzed S-oxidation of **7** were observed (Figure 1).

Reaction of *trans*-2-(4-Nitrophenyl)-1,3-oxathiolane *S*-Oxide with Hydrogen Peroxide. The reaction of excess H_2O_2 with the chemically synthesized *trans*-2-(4-nitrophenyl)-1,3-oxathiolane *S*-oxide diastereomer, **14a**, is a pseudo-first-order process and at neutral pH leads to 4-nitrobenzaldehyde as the sole product. The second-order rate constant for oxidation of **14a** was determined from a plot of k_{obs} vs H_2O_2 concentration (0–464 mM). The plot was linear and had a zero intercept and an excellent correlation coefficient, *r*, of 0.99. In contrast to the oxidation of **7** with H_2O_2, the oxidation of **14a** with H_2O_2 was markedly pH-dependent. In the pH range between 3 and 6.5, the rate of oxidation of **14a** was very slow. In acidic or basic pH regions, a pronounced rate enhancement of the decomposition of the putative *S*,*S*-dioxide of **7** was observed. The only product from oxidation of **14a** in either acidic or basic solution is 4-nitrobenzaldehyde. Since **14a** is relatively hydrolytically stable under the conditions of these experiments (i.e., pH values between 1 and 3.5 and pH values between 6.5 and 9), 4-nitrobenzaldehyde must have arisen from a reaction of acid or base with an oxidation product of **14a** and H_2O_2.

Effects of Para Substituents on the Oxidation of 2-Aryl-1,3-oxathiolane and *trans*-2-Aryl-1,3-oxathiolane *S*-Oxide with H_2O_2. The effect of para substituents on the oxidation of 2-aryl-1,3-oxathiolanes (**1–7**) was determined. In all cases, excellent pseudo-first-order plots were obtained, from which second-order rate constants were determined as described above. Figure 2 shows the results of the oxidation of **1** and **3–7** with sodium periodate and **2–7** with H_2O_2. For both reactions, linear Hammett correlations are apparent.[33] As expected, the rate of oxidation in the presence of $NaIO_4$ was observed to be much faster than for H_2O_2. As the rate of oxidation decreases from one reaction to another, the ρ value becomes larger and more negative (i.e., the ρ values for the oxidation of **1** and **3–7** with $NaIO_4$ and **2–7** with H_2O_2 are −0.22 and −0.34, respectively).

The Hammett plot for the oxidation of para-substituted *trans*-2-aryl-1,3-oxathiolanes *S*-oxides, **10a–14a** by H_2O_2 is markedly steeper; the ρ value is −0.64 and it is apparent that the relative rate of the reaction is quite low (Figure 3).

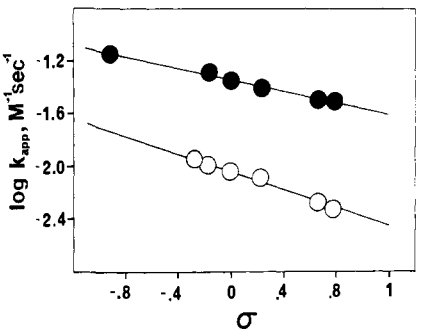

Figure 2. Hammett plot of the apparent second-order rate constants for the oxidation of para-substituted 2-aryl-1,3-oxathiolanes with H_2O_2 (○) and sodium periodate (●) versus σ at pH 7.0.

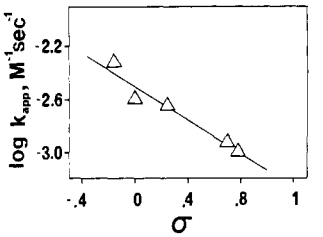

Figure 3. Hammett plot of the apparent second-order rate constants for the oxidation of para-substituted *trans*-2-aryl-1,3-oxathiolane *S*-oxides (compounds **10a–14a**) with H_2O_2 versus σ at pH 7.0.

Reaction Products of the Oxidation of Para-Substituted *trans*-2-Aryl-1,3-oxathiolane *S*-Oxides with Hydrogen Peroxide. Preparative-scale reactions of H_2O_2 with **10a–14a** were performed. In all cases the only organic products extracted from incubations at pH 7.0 were the corresponding para-substituted benzaldehydes, which were identical with authentic samples by TLC and UV–vis spectra.

Microsomal and Highly Purified FMO Mediated Metabolism of Para-Substituted 2-Aryl-1,3-oxathiolanes. The metabolism of **2–7** was studied in vitro. Preliminary studies showed that untreated rat and hog liver microsomes supplemented with NADPH catalyze rapid oxidation of para-substituted 2-aryl-1,3-oxathiolanes to the corresponding *S*-oxides. The formation of *S*-oxide was a linear function of protein concentration (0–3.5 mg/mL). *S*-Oxide formation was linear for at least 8 min except for the formation of **9** and **14**, which was a linear function of time for at least 5 min. Formation of *S*-oxide was temperature dependent (Table I). Heat inactivation of the suspension of hog liver microsomes, under conditions which destroy the flavin-containing monooxygenase (FMO) but not cytochrome P-450[1,20,34] (Table I), completely abolishes *S*-oxide formation, except for compound **4**. In strong contrast, heat inactivation of rat liver microsomes reduces but does not completely abolish *S*-oxide formation. *n*-Octylamine-treated microsomes cause an increase in the formation of the *S*-oxide

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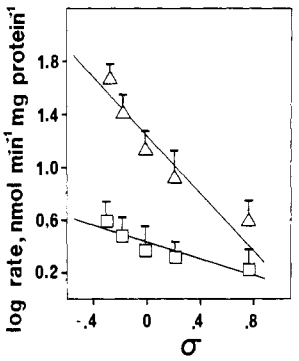

Figure 4. Hammett plot of the rate of *S*-oxidation of para-substituted 2-aryl-1,3-oxathiolanes versus σ with purified hog (△) and rat (□) liver flavin-containing monooxygenase. Each value represents the average of five to seven determinations.

between 3 and 6.6-fold (hog) and greater than 1.9-fold (rat) (Table I), and in the presence of this primary alkylamine the *S*-oxide was the only metabolite detected in dichloromethane extracts analyzed by HPLC.

These results suggest that, at least for hog liver microsomes (with the exception of compound **4**), S-oxidation is catalyzed largely by the FMO since *n*-octylamine is a good inhibitor of cytochrome P-450[35] and a known positive effector for the FMO.[1,14] In rat liver microsomes, S-oxidation is catalyzed at least in part by cytochrome P-450, since heat inactivation of microsomes does not completely abolish S-oxidation (Table I).

Authentic trans *S*-oxides (i.e., compounds **10a–14a**) added to the normal microsomal incubation reaction mixture and immediately extracted were recovered unchanged in greater than 96% yield as judged by HPLC. These results suggest that enzymatic or nonenzymatic reduction of the *S*-oxide produced in microsomal incubations is not occurring. These results also suggest that the relative configurational stereochemistry of the *S*-oxide is unchanged during the course of the metabolic reaction. That the *S*-oxides are quantitatively recovered also suggests that enzymatic or nonenzymatic configurational racemization of the *S*-oxide metabolite is not occurring during the isolation and quantitation procedure; see below.

We determined the effect of substituents on the oxygenation of **2–5** and **7** with highly purified hog and rat liver FMO. Figure 4 shows the results of this study in a typical Hammett fashion. For both enzymes, good linear correlations are apparent. The ρ values for the oxidation of **2–5** and **7** by hog and rat liver FMO are −1.10 and −0.32, respectively. As shown in Table I, no apparent Hammett substituent effect was observed for the microsomal metabolism of **2–5** and **7**. This may be the result of a contribution from cytochrome P-450-mediated S-oxidation which apparently is sensitive to both the lipophilicity and the electronegativity of the para substituent.

Effect of Para Substituents on Microsomal and Highly Purified FMO Mediated Oxidation of *trans*-2-Aryl-1,3-oxathiolane *S*-Oxide. The effect of para substituents on the microsomal metabolism of **10a–13a** and **14a** was investigated in vitro. The transformation of the major trans diastereomer of the chemically synthesized para-substituted 2-aryl-1,3-oxathiolane *S*-oxides with hog and rat liver microsomes supplemented with NADPH to produce the corresponding benzaldehyde was linearly dependent on protein concentration and with incubation time for at least 10 min. *n*-Octylamine-treated microsomes catalyzed the disappearance of the *S*-oxide more than 2-fold and heat inactivation of both rat and hog liver microsomes completely abolished para substituted benzaldehyde formation (data not shown).

Figure 5 shows the results of the enzymatic and microsomal metabolism of para-substituted *trans*-2-aryl-1,3-oxathiolane *S*-oxides in a typical Hammett plot. For both highly purified FMO and microsomal transformations, good linear correlations are

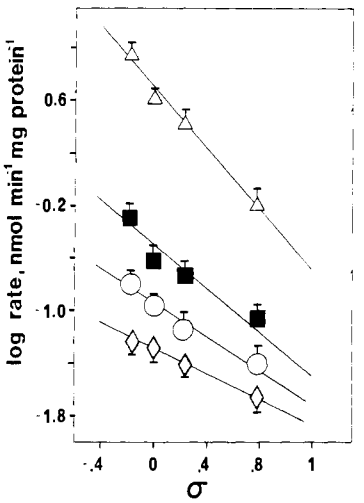

Figure 5. Hammett plot of the rate of S-oxidation of para-substituted *trans*-2-aryl-1,3-oxathiolane *S*-oxides (compounds **10a–12a** and **14a**) with purified hog (△) and rat (■) liver FMO and hog (○) and rat (◇) liver microsomal flavin-containing monooxygenase versus σ. Each value represents the average of five to seven determinations.

Table II. Hammett Correlations for S-Oxidation

reactant	substrate	ρ value[a]	ref
$NaIO_4$	oxathiolanes	−0.22	this study
H_2O_2	oxathiolanes	−0.34	this study
hog FMO[b]	oxathiolanes	−1.10	this study
rat FMO	oxathiolanes	−0.32	this study
hog FMO	oxathiolane *S*-oxides	−1.23	this study
rat FMO	oxathiolane *S*-oxides	−0.84	this study
hog microsomes[b]	oxathiolane *S*-oxides	−0.65	this study
rat microsomes	oxathiolane *S*-oxides	−0.49	this study
H_2O_2	thioanisoles	−1.17	37
$NaIO_4$	thioanisoles	−1.40	38
4a-peroxyflavin	thioanisoles	−1.68	9
cytochrome P-450	thioanisoles	−0.14	41

[a] The ρ values are calculated from plots of log rate vs σ. [b] Refers to hepatic FMO or hepatic microsomes.

apparent. The ρ values for the oxidation of **10a–13a** and **14a** by highly purified hog and rat liver FMO and hog and rat liver microsomes are −1.23, −0.84, −0.65, and −0.49, respectively, Table II. It is apparent that in spite of at least a 130-fold decrease in rate of highly purified hog liver FMO catalyzed S-oxidation, the ρ value for oxidation of **10a–13a** and **14a** does not become significantly larger and more negative compared to the oxidation of **3** to **7**. However, for oxidation of **10a–13a** and **14a** with highly purified rat liver FMO, the ρ values becomes progressively larger and more negative. These results suggest that the highly purified rat liver FMO is considerably less efficient at S,S-dioxygenation and consequently more sensitive to electronic substituent effects than the highly purified hog liver FMO. The effects of para substitution is most pronounced for the putative S,S-dioxygenation step in the overall metabolism of aryl-1,3-oxathiolane to benzaldehyde (Scheme I). This finding is similar to results observed for the microsomal S-oxidation of para-substituted thiobenzamide *S*-oxides.[36] From the reactivity–selectivity principle and the lower order of reactivity of 2-aryl-1,3-oxathiolane *S*-oxides versus the 2-aryl-1,3-oxathiolanes to oxidizing agents, a much greater sensitivity of substituents to oxygenation is anticipated.

Diastereoselectivity of Sulfoxidation Catalyzed by Hog and Rat Liver Flavin-Containing Monooxygenase. With the exception of 2-(4-nitrophenyl)-1,3-oxathiolane, compound **7**, the S-oxidation of **2–5** by highly purified hog liver FMO is a highly stereoselective process. In all cases examined, the major enzymatic diastereomer is identical with the major trans diastereomer formed via chemical oxidation of **2–7**. For compounds **2–5**, the major trans diaste-

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Table III. Stereoselectivity of S-Oxygenation Catalyzed by Hog Liver Flavin-Containing Monooxygenase

2-aryl-1,3-oxathiolane		percent S-oxide diastereomer formed							
		pure FMO: comp[a]		microsomes					
				comp[a]		-NOA[b]		+heat inactiv[c]	
compd	aryl	cis[d]	trans[d]	cis	trans	cis	trans	cis	trans
2	4-CH_3O	7.0	93.0	7.2	92.8				
3	4-CH_3	1.0	99.0	12.2	83.0	4.6	95.4	ND[e]	ND[e]
4	4-H	8.0	92.0	42.0	57.0	14.0	86.0	26.0	74.0
5	4-Cl	2.6	97.4	13.3	86.6	3.5	95.5	ND[e]	ND[e]
7	4-NO_2	24.0	76.0	33.0	67.0	9.5	90.5	ND[e]	ND[e]

[a] The complete system contained 50 mM phosphate buffer, pH 7.8, the NADPH generating system, 200 mM substrate, 4 mM *n*-octylamine, and 0.066 mg FMO or 0.56 mg of microsomal protein incubated for 3 min at 33 °C as described in the Experimental Section. Data represent the average of three determinations. [b] Complete system without *n*-octylamine (NOA). [c] Complete system without *n*-octylamine pretreated with heat inactivation as described in the Experimental Section. Heat-inactivated microsomes used in this study retained 85–100% of their cytochrome P-450 activity (ref 29). [d] Cis and trans refer to the configuration of the *S*-oxide diastereomers as determined by HPLC. [e] Not detectable.

Table IV. Stereoselectivity of S-Oxygenation Catalyzed by Rat Liver Flavin-Containing Monooxygenase

2-aryl-1,3-oxathiolane		percent S-oxide diastereomer formed							
		pure FMO: comp[a]		microsomes					
				comp[a]		-NOA[b]		+heat inactiv[c]	
compd	aryl	cis[d]	trans[d]	cis	trans	cis	trans	cis	trans
2	4-CH_3O	33.0	66.0	20.0	80.00				
3	4-CH_3	4.8	95.2	11.0	89.0	3.0	97.0	2.0	98.0
4	4-H	6.0	94.0	13.2	86.7	5.5	94.5	3.0	97.0
5	4-Cl	10.0	90.0	21.8	78.2	7.0	93.0	7.5	92.5
7	4-NO_2	22.0	78.0	13.5	86.5	5.3	94.7	0.5	99.5

[a] The complete system contained 50 mM phosphate buffer, pH 7.8, the NADPH generating system, 200 mM substrate, 4.0 mM *n*-octylamine, and 0.68 mg FMO or 0.87 mg of microsomal protein incubated for 5 min at 33 °C as described in the Experimental Section. Data represent the average of three determinations. [b] Complete system without *n*-octylamine (NOA). [c] Complete system without *n*-octylamine pretreated with heat inactivation as described in the Experimental Section. Heat-inactivated microsomes used in this study retained 80–100% of their cytochrome P-450 activity (ref 29). [d] Cis and trans refer to the configuration of the *S*-oxide diastereomers as determined by HPLC.

reomer was formed with high selectivity (92–99% of the *S*-oxide produced is one diastereomer) (Table III). In contrast to the results from highly purified FMO, microsomal metabolism of **3–5** and **7** gave *S*-oxide metabolites of lower configurational purity. Incubation of **2–5** and **7** with hog liver microsomes in the absence of *n*-octylamine actually improved the configurational purity of the *S*-oxide metabolites observed to be formed with microsomal preparations. In cases where hog liver microsomes were pretreated with heat under conditions which completely abolish FMO activity and retain cytochrome P-450 activity,[29] no detectable S-oxidation of 2-aryl-1,3-oxathiolanes was observed except for compound **4**. The results of these studies with hog liver FMO and hog liver microsomes suggest that S-oxidation of 2-aryl-1,3-oxathiolanes is almost entirely due to FMO activity. For compound **4**, cytochrome P-450 contributes to S-oxidation and carries out oxygenation in a way that decreases the stereoselectivity of the microsomal reaction.

For highly purified rat liver FMO, S-oxygenation of **3–5** is a highly stereoselective process with the major trans diastereomer being formed in large excess (i.e., 90–95%) (Table IV). In agreement with the results observed for hog liver FMO activity, the major *S*-oxide diastereomer produced is identical with the major trans *S*-oxide diastereomer formed by chemical oxidation of **2–5** and **7**. S-oxygenation of compounds **2** and **7** with highly purified rat liver FMO demonstrated the lowest degree of stereoselectivity of the compounds tested. In contrast to highly purified rat liver FMO, the microsomal metabolism of **2** and **7** produced *S*-oxides with much higher configurational purity. Surprisingly, the S-oxidation of **3–6** with rat liver microsomes in the absence of *n*-octylamine gave products with great configurational purity. In cases where microsomes were pretreated with heat, a low but significant amount of S-oxidation was observed (i.e., see Table I) and the *S*-oxides produced were of high configurational purity (Table IV). These results are best explained by suggesting that cytochrome P-450 also contributes to rat liver microsomal S-oxidation of **2–5** and **7**. In contrast to hog liver microsomal cytochrome P-450, however, where S-oxidation of **4** is observed to give greater amounts of the minor cis diastereomer, for rat liver microsomal cytochrome P-450, the product is formed with high configurational purity and is identical with the product arising from highly purified rat liver FMO.

It is clear from our studies and others[30–33] that the major diastereomer produced by chemical and enzymatic oxidation of **1–7** has the oxygen on sulfur and the aryl group in a trans arrangement; see Scheme I. Attempts to demonstrate that the enzyme oxidizes sulfur in an enantioselective fashion at the *pro-R* position as in the case of 4-tolylethylsulfide[23] has not been possible due to the inability to separate (*R*)- and (*S*)-*trans*-2-aryl-1,3-oxathiolane *S*-oxides on chiral stationary phase HPLC columns,

Discussion

Chemical Oxidation of 2-Aryl-1,3-oxathiolanes. The reaction of **1–7** with excess H_2O_2 or sodium periodate was investigated to identify the mechanism of the reaction and the products formed. H_2O_2 was chosen as the oxidant because the FMO enzyme generates a 4a-hydroperoxyflavin as the enzymatic oxidant.[1,3–7] 2-Aryl-1,3-oxathiolanes are rapidly oxidized to 2-aryl-1,3-oxathiolane *S*-oxides, **8–14**, which are then slowly converted to benzaldehydes (Scheme I). The conversion of **9–14** to benzaldehydes must involve an oxidative step, since in the absence of H_2O_2 this reaction is extremely slow; i.e., at pH 7.4 the half-life for conversion of **14a** to 4-nitrobenzaldehyde is 960 h, whereas in the presence of 0.22 M H_2O_2 the half-life is 39 h. The ultimate formation of benzaldehyde is attributed to the hydrolysis of the highly unstable *S,S*-dioxide. Kinetic studies showed that the reaction of **2–7** with excess H_2O_2 or **1** and **3–7** with excess sodium periodate is pseudo first order and is not subject to specific-acid or buffer catalysis in the range from pH 1 to 7 (Figure 1). The second-order rate constants obtained from these pseudo-first-order studies indicated that those 2-aryl-1,3-oxathiolanes with electron-donating substituents were oxidized faster (Figure 2). The ρ values obtained for the oxidation with sodium periodate and H_2O_2 are −0.22 and −0.34, respectively. These values may be compared with the ρ values of −1.17 and −1.40 reported for the oxidation of para-substituted thioanisoles with H_2O_2[37] and sodium periodate,[38] respectively (Table II). The results of Figure 2 show

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that the rate of the oxidation of **1–7** is faster for the oxyanion sodium periodate than for H_2O_2 in accordance with the overall participation of base (Figure 1) in the S-oxidation of 2-aryl-1,3-oxathiolanes. The S-oxidation of **7** was examined in some detail because preliminary studies had demonstrated that the corresponding trans *S*-oxide, **14a**, was extremely stable and possessed excellent properties suitable for convenient analysis. Kinetic studies of **7** showed that between the range from pH 3 to 9 the hydrolysis was extremely slow (i.e., the half-life value was greater than 41 days). However, in the presence of H_2O_2, the oxidation of **7** was significantly accelerated in the presence of base.

Chemical Oxidation Studies of Para-Substituted *trans*-Aryl-1,3-oxathiolane *S*-Oxides. Kinetic studies of the oxidation of **14a** with excess H_2O_2 demonstrated that the reaction was pseudo first order. In contrast to the oxidation of **7**, the overall oxidation reaction is slower for **14a** and the apparent second-order rate constant increases rapidly with acid and base (Figure 1). Since this reaction is also not subject to buffer catalysis, the increase in rate must be attributed to specific-acid and specific-base catalysis. This suggests that the reactants protonate or deprotonate to form highly reactive species which produce 4-nitrobenzaldehyde in a very fast subsequent step. As discussed above, **9a–14a** are relatively stable in the absence of H_2O_2 in the range from pH 3 to 9.

Kinetic studies of the reaction of **10a–14a** with excess H_2O_2 demonstrated pseudo-first-order reaction kinetics. The apparent second-order rate constants obtained from replots of k_{obs} versus H_2O_2 indicate that those 2-aryl-1,3-oxathiolane *S*-oxides with electron-donating substituents were oxidized faster than ones with electron-withdrawing substituents (Figure 3). The ρ value obtained was −0.64. The data of Figure 3 suggest that H_2O_2 serves as an electrophilic oxidizing agent and, in contrast to oxidation of **1–7**, the overall sensitivity of the reaction to the substituent becomes greater with the less reactive *S*-oxides, **10a–14a**. Although H_2O_2 serves as an electrophilic reagent at pH 7, there is literature precedent for HOO^- to serve as a nucleophilic oxidizing agent at higher pH.[18,39,40]

Effect of Para Substituents on the Microsomal S-Oxidation of 2-Aryl-1,3-oxathiolanes. The oxidative transformation of **2–7** was investigated in vitro with hog and rat liver microsomes. Results shown in Table I indicate that 2-aryl-1,3-oxathiolanes are rapidly and extensively converted to their *S*-oxides by microsomes supplemented with oxygen and an NADPH-generating system. That *S*-oxide formation is dependent in large part upon FMO is apparent since (1) activity is stimulated by *n*-octylamine, a known activator of FMO[1] and an inhibitor of cytochrome P-450[35] and (2) activity is absent in microsomes heat treated under conditions which inactivate FMO but leave cytochrome P-450 active.[29] However, the data provide evidence that *S*-oxidation of **4** (in hog liver microsomes) and **3–7** (in rat liver microsomes) have a contribution from cytochrome P-450 (Table I). Clearly, however, FMO-catalyzed oxidation represents by far the major pathway for *S*-oxide formation in hog and rat liver microsomes. The lack of Hammett correlations with microsomal metabolic S-monooxidation could potentially arise from a minor contribution of S-oxidation from cytochrome P-450 and the lipophilic nature of the microsomal monooxygenase system or from the relative nucleophilicity of the substrate. As shown in Table I, the lipophilic primary alkylamine *n*-octylamine stimulates FMO-mediated S-oxygenation, but in its absence S-oxygenation does not yield a Hammett correlation either.[41] As discussed below, the involvement of cytochrome P-450 can be more clearly seen by an investigation of the stereochemical outcome of microsomal S-oxidation.

Stereoselectivity of S-Oxidation Catalyzed by Microsomal and Highly Purified Flavin-Containing Monooxygenase. Hog liver FMO has been shown to S-oxidize dialkyl sulfides with 93–95% stereospecificity in a process where the minor *S*-oxide enantiomer arises due to incomplete chiral processing by the enzyme and not to a competing, achiral nonenzymatic sulfoxidation process.[23] In addition, it was shown that the phenobarbital-induced rat liver cytochrome P-450 preferentially catalyzed formation of the opposite *S*-oxide enantiomer as that formed by FMO.[42] Other studies have demonstrated that FMO-mediated oxidation of the dialkyl sulfide disulfoton is stereoselective since the *S*-oxide product is optically active.[24]

The diastereoselectivity of sulfoxidation of **2–5** and **7** catalyzed by hog and rat liver microsomes and highly purified hog and rat liver FMO was determined (Tables III and IV). As shown in Tables III and IV, highly purified hog and rat liver FMO S-oxidize 2-aryl-1,3-oxathiolanes with a varying degree of configurational purity. No apparent relationship between electronic substituent character and stereoselective enzymatic processing was observed. In general, however, more lipophilic substrates are S-oxidized with a lower degree of stereoselectivity. For hog liver microsomes, S-oxidative processing of **3–5** and **7** was less selective in the presence of *n*-octylamine. Heat inactivation completely abolished S-oxidation except for **4**, which demonstrated the lowest degree of stereoselectivity of the compounds examined. We conclude that **4** is S-oxidized to an appreciable extent with hog liver microsomal cytochrome P-450 and because cytochrome P-450 is only modestly stereoselective, the minor diastereomer produced is mostly formed via cytochrome P-450 mediated S-oxidation. As shown in Table IV, S-oxidation of **2–5** and **7** with rat liver FMO was less stereoselective than that with hog liver FMO. In agreement with results from hog liver FMO, the stereoselective sulfoxidation catalyzed by rat liver FMO showed no apparent dependence on the electronic nature of the para substituent. In general, microsomal S-oxidation was less stereoselective than that with the purified enzyme, but surprisingly, in all cases examined, a modest increase in stereoselective processing was observed for microsomal S-oxidation in the absence of *n*-octylamine. That the heat inactivated microsomes show the greatest degree of stereoselectivity suggests that (1) rat liver cytochrome P-450 contributes to S-oxidation of **3–5** and **7**, (2) rat liver microsomal cytochrome P-450 S-oxidation gives the same major trans *S*-oxide product as rat liver FMO, or (3) a second, relatively heat-stable form of FMO is present in rat liver microsomes which metabolizes **3–5** and **7** with the same stereoselectivity as the heat-labile form of FMO. The possibility that two forms of mouse liver FMO exist has been previously suggested.[28] It may be that two forms of rat liver FMO also exist, one of which is relatively heat sensitive and one of which is not heat sensitive.

One possible explanation for the lack of diastereoselective purity for FMO-catalyzed S-oxidation could be that a concurrent nonenzymatic S-oxidation contributes to the formation of the minor diastereomer. That this is not the case is apparent since (1) the ratio of diastereomers remains constant over the time course of the reaction, (2) in the case of chemical oxidation, enormous concentrations of H_2O_2 (i.e., 100–200 mM) are required to effect the same degree of S-oxidation observed for the enzymatic cases, and (3) metabolic incubations of **2–7** in the presence of glutathione did not appreciably alter the stereoselectivity of S-oxidation (data not shown).

Effect of Para Substituents on the S-Oxidation of 2-Aryl-1,3-oxathiolanes with Purified Flavin-Containing Monooxygenase. The transformation of **2–5** and **7** with purified hog and rat liver FMO supplemented with NADPH was investigated in vitro. During the short incubation times employed, dichloromethane extracts of metabolic reactions produced a single product which coeluted by HPLC with the corresponding trans *S*-oxide. As shown in Figure 4, **2–5** and **7** are excellent substrates for both the purified hog and rat liver FMO. In agreement with previous reports, hog

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liver FMO S-oxidizes dialkyl sulfides more efficiently than rat liver FMO.[42] In contrast to the results obtained from microsomal preparations, however, purified FMO from both sources S-oxidize **2–5** and **7** in a process dependent on the para substituent. Studies with 2-aryl-1,3-oxathiolanes indicated that those with electron-donating substituents were oxidized faster (Figure 4). The ρ values obtained for the oxidation of 2-aryl-1,3-oxathiolanes with rat and hog liver FMO are −0.3 and −1.10, respectively.

These values may be compared with the ρ value of −0.22 and −0.34 for the oxidation of para-substituted thioanisoles discussed above (Table II) by H_2O_2 and sodium periodate, respectively.[37,38] While no clear-cut distinction between the size of the ρ value and the degree of nucleophilic versus radical character can be drawn from this data, for FMO we favor a mechanism involving nucleophilic character. In contrast, cytochrome P-450 catalyzed oxidation of dialkyl sulfides[43] (see Table II) or amines[44,45] has been demonstrated to possess a great deal of single electron transfer character.

Effect of Para Substituents on the S-Oxidation of *trans*-2-Aryl-1,3-oxathiolane *S*-Oxides. The transformation of **10a–14a** was carried out with purified FMO enzyme and microsomal preparations from hog and rat liver. The only metabolite detected from dichloromethane extracts of biotransformation reactions of **10a–14a** coeluted with the corresponding authentic benzaldehydes. The para-substituted benzaldehydes were also isolated and purified. The UV–vis spectra of the benzaldehydes formed by metabolic reactions as well as their phenylhydrazones were identical with authentic materials. That the S-oxidation of **10a–14a** was due to FMO and not cytochrome P-450 comes from preliminary studies which indicated that oxidation of 2-aryl-1,3-oxathiolane *S*-oxides is dependent on NADPH and O_2 and stimulated 1–3-fold by *n*-octylamine. Heat inactivation of the enzyme preparation abolishes benzaldehyde formation (data not shown). As shown in Figure 5, **10a–14a** were oxidized by FMO at a significantly lower rate than their corresponding sulfides. In addition, the S-oxidation of *trans*-2-aryl-1,3-oxathiolane *S*-oxides is clearly dependent on the electronic properties of the para substituent: those with electron-donating substituents were oxidized markedly faster than those with electron-withdrawing substituents. The ρ values obtained for the oxidation of *S*-oxides with rat or hog liver microsomes and purified rat or hog liver FMO are −0.49, −0.65, −0.84, and −1.23, respectively. The idea that further oxidation of *S*-oxides involves nucleophilic attack on electrophilic FMO peroxyflavin may help to explain the increase in sensitivity of the reaction to para substituents. It is possible that the low reactivity of S-oxides versus the parent sulfide exacerbates the substituent effect and the ρ values become progressively larger and more negative. We interpret the data to suggest that the FMO-mediated oxidation of sulfoxides has even a greater degree of nucleophilic character than the oxidation of the parent sulfides.

Nucleophilic attack by sulfides on electrophilic peroxides may occur by either a nucleophilic reaction or by a radical-mediated process initiated by a single electron transfer (SET) reaction. Although the data presented herein and elsewhere[9–12,16,17,39–41] are consistent with a nucleophilic process for peroxyflavin-mediated S-oxygenation, Hammett-type correlations are not sufficient to unambiguously distinguish SET reactions from nucleophilic displacement reactions.[16] However, by an analysis of the stereochemical integrity of the products of such reactions, it may be possible to assess the contribution of a sulfide radical cation to the reaction process. As discussed above, the high stereoselective nature of the enzymatic reactions does not suggest a significant involvement of radical intermediates. In addition, the lack of materials derived from benzylic carbon radical products also suggests that radical species are not present. However, the lack of products derived from a mechanism involving radicals does not preclude the possibility of radical character in the reaction. In cases of microsomal reactions in which low configurational purity of products is observed, we suggest that cytochrome P-450 may contribute to S-oxygenation. The mechanism of cytochrome P-450 mediated S-oxygenation presumably has a great deal of SET character[44,45] and this could account for the modest stereoselectivity observed. It is also possible that SET processes contribute to FMO-catalyzed reactions. For example, it is possible that in the presence of the primary alkylamine *n*-octylamine, an agent which increases the rate of FMO, S-oxygenation by SET processes may make a contribution to the overall reaction.

We interpret our results as an example of the reactivity–selectivity principle of FMO action. For FMO substrates with modest nucleophilicity, the rate-determining step could manifest itself as rate-limiting nucleophilic attack upon electrophilic peroxyflavin. For forms of FMO (such as rat liver FMO) which are insensitive to the nucleophilicity of even modest nucleophiles, other steps in the enzyme reaction must be rate determining. Another possibility is that more than one form of the same enzyme is present.

Conclusions

The overall reaction of 2-aryl-1,3-oxathiolanes with H_2O_2 or the flavin-containing monooxygenase can be described by Scheme I, in which each step has been investigated chemically as well as enzymatically and represents a process which converts 2-aryl-1,3-oxathiolanes to benzaldehydes. In both the chemical and enzymatic S-oxygenation, the second oxygenation is much slower than the first and demonstrates a much greater sensitivity to para substituents. Compared to hog liver, the rat liver flavin-containing monooxygenase was less efficient at 2-aryl-1,3-oxathiolane S-oxygenation and much less sensitive to para substituents. These results suggest that different forms of the enzyme may be present in rat liver and hog liver and/or that some other step in the S-oxygenation of dialkylsulfides may be partially rate limiting for the two enzyme forms.

The stereochemical outcome of the S-oxygenation of 2-aryl-1,3-oxathiolanes was investigated by highly purified and microsomal hog and rat liver flavin-containing monooxygenase. With the exception of **7**, 2-aryl-1,3-oxathiolanes produce one major trans *S*-oxide diastereomer with good stereoselectivity (i.e., 93–99%). The hog liver microsomal metabolism of 2-aryl-1,3-oxathiolanes had similar stereoselectivity as the purified enzyme with the exception of **4** and this was apparently due to an involvement of cytochrome P-450 mediated S-oxygenation. In contrast, the rat-liver flavin-containing monooxygenase was only modestly stereoselective. S-oxygenation of 2-aryl-1,3-oxathiolanes with rat liver microsomes demonstrated that a heat-stable form of the flavin-containing monooxygenase and/or cytochrome P-450, both with similar stereoselectivity, contributed to S-oxygenation.

The S-oxygenation of para-substituted *trans*-2-aryl-1,3-oxathiolane *S*-oxides with H_2O_2 or highly purified and microsomal hog and rat liver flavin-containing monooxygenase is considerably slower and more sensitive to para substituents than the parent sulfides in accordance with the reactivity–selectivity principle.[46] The S-oxygenation of para-substituted *trans*-2-aryl-1,3-oxathiolane *S*-oxides is performed almost exclusively by the flavin-containing monooxygenase with very little involvement from cytochrome P-450 mediated oxidation. The product of the reaction is the corresponding benzaldehyde which arises from hydrolysis of the unstable 2-aryl-1,3-oxathiolane *S,S*-dioxide. That the H_2O_2-mediated oxygenation of para-substituted *trans*-2-aryl-1,3-oxathiolane *S*-oxides is similar to that of the purified enzyme and gives negative ρ values suggests that the mechanisms are similar and involve attack of the *S*-oxide sulfur on electrophilic peroxide.

Experimental Section

Chemicals. All chemicals used were reagent grade or better and all compounds listed in Tables V and VI were fully characterized by IR, UV–vis, NMR, and mass spectrometry. The 2-aryl-1,3-oxathiolanes

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Table V. Properties of Aryl-1,3-oxathiolane *S*-Oxides

S-oxide diastereomer	aryl	NMR[a] H(1)	NMR[a] H(2)	NMR[a] H(3)	high-resolution mass spectra[b] calcd	high-resolution mass spectra[b] obs	high-resolution mass spectra[b] ±ppm
9a	4-OCH_3	4.5–4.8 (7)	2.8–3.1 (m)	5.5 (s)	212.0509	212.0510	+1.6
9b	4-OCH_3	4.5–4.7 (m)	3.1–3.7 (m)	5.3 (s)	212.0509	212.0506	−0.6
10a	4-CH_3	4.5–4.8 (m)	2.8–3.3 (m)	5.5 (s)	196.0557	196.0557	−0.3
11a	4-H[c]	4.5–4.8 (m)	2.8–3.1 (m)	5.5 (s)	182.0404	182.0405	+1.9
12a	4-Cl	4.5–4.8 (m)	2.9–3.1 (m)	5.5 (s)	216.9975	216.9976	+0.5
12b	4-Cl	4.4–4.9 (m)	2.8–3.2 (m)	5.5 (s)	216.9975	216.0005	−3.3
13a	4-CN	4.4–5.0 (m)	2.6–3.3 (m)	5.5 (s)	207.0358	207.0359	+2.6
13b	4-CN	4.9–5.2 (m)	3.0–3.7 (m)	5.5 (s)	207.0358	207.0354	+0.1
14a	4-NO_2	4.4–5.0 (m)	2.8–3.1 (m)	5.5 (s)	227.0261	227.0262	+4.1
14b	4-NO_2	4.3–4.9 (m)	3.0–3.7 (m)	5.5 (s)	227.0261	227.0262	+4.3

[a] NMR were taken on a FT-80 MHz instrument employing TMS on an internal standard. H(1) are the protons adjacent to oxathialone oxygen, H(2) are the protons adjacent to oxathiolane sulfur, and H(3) is the benzylic proton. [b] Mass spectrometry was obtained with a modified Kratos MS-25 in the electron-impact mode. [c] These results are similar to previously reported values (ref 30).

Table VI. Properties of 2-Aryl-1,3-oxathiolanes and Their *S*-Oxides

compd	aryl	mp, °C[a]	ret vol, mL[b]	$\lambda_{max}(\epsilon)$[c]
		2-Aryl-1,3-oxathiolanes		
1	4-OH	92–94	3.53, A	230 (17 250)
2	4-CH_3O	35–37	6.05, A	230 (10 250)
3	4-CH_3	*d*	8.26, A	240, sh (3 600)
4	4-H	*d*	6.36, A	203 (8 750)
5	4-Cl	*d*	9.24, A	220 (12 000)
6	4-CN	37–39	6.68, A	231 (17 500)
7	4-NO_2	73–75	6.15, A	266 (9 750)
		2-Aryl-1,3-oxathiolane *S*-Oxide Diastereomers		
8a,[f] **b**[g]	4-OH	*e*	2.54,[f] 2.17,[g] B	236,[f] 239[g] (14 500, 15 300)
9a,b	4-CH_3O	*d*	3.23, 3.03, B	234, 235 (11 500, 12 350)
10a,b	4-CH_3	*d*	3.71, 3.31, B	223, 225 (11 750, 12 050)
11a,b	4-H	*d*	3.26, 3.03, B	214, 215 (7 600, 7 950)
12a,b	4-Cl	*d*	3.90, 3.40, B	227, 228 (14 850, 15 600)
13a,b	4-CN	133–135, 168 (d)	3.85, 3.47, B	236, 234 (16 750, 17 750)
14a,b	4-NO_2	114–116, 165 (d)	3.26, 3.06, B	270, 272 (8 750, 9 750)

[a] Melting points were taken in a Melt Temp apparatus and are uncorrected. [b] Retention volumes were measured on RPHPLC as described in the Experimental Section; solvent A is CH_3CN/H_2O (66:33) and solvent B is CH_3CN/H_2O (53:46). [c] UV spectra were recorded in methanol; λ (ε) values are in units of nm (M^{-1} cm^{-1}). [d] These materials are oils. [e] This material is quite unstable. [f] Major trans diastereomer, compound **a**. [g] Minor cis diastereomer, compound **b**.

were synthesized by using the method of Jaeger and Smith.[47] 2-Aryl-1,3-oxathiolane *S*-oxides were prepared by treating a methanolic solution of the 2-aryl-1,3-oxathiolane with 1 equiv of sodium periodate at room temperature until TLC indicated disappearance of the starting materials. The properties of these materials are shown in Table V. After filtration and removal of the solvent under vacuum, the crude *S*-oxides were purified by preparative TLC (silica gel, 500 μm, methanol/dichloromethane, 1:99, three to five developments) to afford pure *S*-oxide diastereomers which were recrystallized from acetonitrile. For the chemical synthesis, the ratio of cis to trans *S*-oxide was between 15:85 and 40:60. We cannot account for the instability of the *S*-oxides reported by others.[30] The relative configuration of the trans and cis *S*-oxides (series a and b, Table V, respectively) were assigned by high-field NMR studies and are in agreement with previous studies.[30] High-resolution mass spectrometry of synthetic *S*-oxides (i.e., ±5 ppm of the calculated mass) are in excellent agreement with the predicted structure (Table V). All attempts to separate enantiomers of the purified *S*-oxide diastereomers by chiral stationary phase HPLC were unsuccessful.

Materials. All solvents and buffers were the purest commercially available products. Para-substituted benzaldhydes, 30% aqueous hydrogen peroxide, *n*-octylamine, and EDTA were obtained from Aldrich Chemical Company, Milwaukee, WI. $NADP^+$, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from Sigma Chemical Co.

Measurement of Rate Constants. All rate measurements were made at 37.0 ± 0.2 °C with an IBM Model 9300 Ternary Gradient high-performance liquid chromatography (HPLC) system. Analysis was performed with a 5-μm CRI C_{18} ODS Chromosphere (7.7 mm × 25 cm) analytical reverse-phase column. The mobile phase consisted of acetonitrile/water (66:33, v/v) with UV detection. The buffers employed were HCl (pH <3), acetate (pH 3–6), phosphate (pH 6–8), and borate (pH 8–10); ionic strength was maintained at 1.0 M with KCl. Metal-free conditions were ensured by the addition of 1 mM EDTA. Hydrogen peroxide solutions were standardized by iodometric titration.[48] Stock solutions of 2-aryl-1,3-oxathiolanes and their S-oxides were prepared in dry methanol and aliquots were diluted into the aqueous reaction solutions such that the final concentration of methanol never exceeded 2.0%. Quantitation of amounts of products were performed by comparison with standard curves after correction for extinction coefficients at the wavelength employed. The amount of product was calculated by regression analysis from a standard curve of authentic material under the same analysis conditions. The retention times of the various products are listed in Table VI. Rate constants (k_{obs}) were measured under pseudo-first-order reaction conditions by monitoring the formation or disappearance of *S*-oxide or the formation of para-substituted benzaldehydes for at least 5 half-lives, and second-order rate constants were obtained from plots of k_{obs} versus the concentration of the reactant present in large excess (usually H_2O_2 or $NaIO_4$). Buffer catalysis could not be detected in any of the reactions studied. *S*-Oxide reaction products were determined by comparison with standard curves of authentic materials. Reaction products for the oxygenation of *trans*-2-aryl-1,3-oxathiolane *S*-oxides were determined by large-scale reactions (same concentrations as the kinetic runs) and dichloromethane extraction were followed by preparative TLC. The 2-(4-nitrophenyl)-1,3-oxathiolane **7** was chosen for many of the kinetic studies because of the ease of analyzing the products of oxidation and its hydrolytic stability.

Metabolic and Enzymatic Incubations. Hog liver microsomes were a generous gift of Professor D. M. Ziegler (University of Texas at Austin). Hog liver flavin-containing monooxygenase was purified by using published procedures.[50] Purified hog liver flavin-containing monooxygenase and hog liver microsomes used in this study were shown to N-oxygenate dimethylaniline very efficiently.[15] Rat liver microsomes were isolated by the method described earlier.[49] Rat liver flavin-containing mono-

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oxygenase was purified by using a modified method of Sabourin et al.[50] The incubation medium contained 50 mM potassium phosphate; pH 7.8, 0.5 mM $NADP^+$, 2.0 mM glucose 6-phosphate, 1 IU of glucose-6-phosphate dehydrogenase, 4 mM *n*-octylamine, and 0.5-0.9 mg of microsomes or 65-690 mg of purified flavin-containing monooxygenase as described previously.[51] Reactions were initiated by the addition of the 2-aryl-1,3-oxathiolane or the 2-aryl-1,3-oxathiolane *S*-oxide and the incubation was continued with constant shaking to maintain adequate oxygen concentrations. At timed intervals the reaction was quenched and analyzed for products by the procedures given below.

The reaction was quenched with 1 mL of cold dichloromethane and the aqueous reaction medium was separated by centrifugation. The dichloromethane fraction was filtered through a Sep Pak and then evaporated to dryness and redissolved in 0.5 mL of methanol. The metabolic products were quantitated by HPLC as described above. Quantitation of para-substituted benzaldehydes by HPLC utilized a mobile phase consisting of acetonitrile/water (53:46). HPLC can efficiently separate aryl-1,3-oxathiolanes, aryl-1,3-oxathiolane *S*-oxides, and benzaldehydes.[52] The material balance was 95% as judged by parallel experiments with authentic internal standards.

Heat inactivation of hog or rat liver microsomal protein was accomplished by purging the protein suspended in buffer, pH 7.8, with argon and placing the microsomal protein in a bath of 55 °C water for 60 s in the absence of NADPH. This procedure has been shown to completely destroy FMO-dependent N-oxygenation while preserving 85-100% of cytochrome P-450 mediated N-dealkylation.[29]

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A Stereoselective Synthesis of (-)-Perhydrohistrionicotoxin

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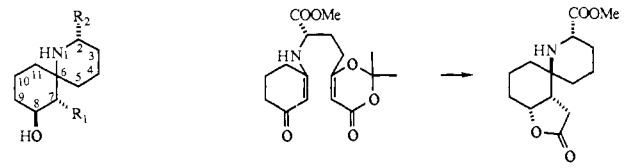
Abstract: The first synthesis of (-)-perhydrohistrionicotoxin, **2**, which proceeds without recourse to resolution of synthetic intermediates is reported. The absolute stereochemistry is derived from L-glutamic acid, and the key step, in which the critical relative stereochemical relationships are established, is the intramolecular photocycloaddition of a vinylogous amide with a dioxenone. The transformation of **5** into **2** proceeds in 16 steps in 9% overall yield.

In 1971, Witkop and co-workers reported the isolation of (-)-histrionicotoxin, **1** (Scheme I), from the skin extracts of the Neotropical poison frog *Dendrobates histrionicus*.[3] The attention given to the synthesis of the histrionicotoxin alkaloids[4] stems from their unique properties as neurotoxins in conjunction with their scarcity (ca. 200 μg per frog). It has been shown that both histrionicotoxin, **1**, and perhydrohistrionicotoxin, **2**, are potent blockers of acetylcholine-mediated ion conductance, thereby interrupting transsynaptic transmission of neuromuscular impulses. Both compounds are therefore of considerable importance in studying cholinergic receptor mechanisms in the neuromuscular system.[5,6]

Scheme I



1 R_1 = HC≡CH-C≡CH; R_2 = CH_2-HC=CH-C≡CH

2 R_1 = C_4H_9; R_2 = C_5H_{11}

3

4

We have recently reported the transformation of **3**, derived from L-glutamic acid, into **4**, which possesses the azaspiroundecane skeleton of (-)-histrionicotoxin, **1**.[7] The key step in the conversion of **3** → **4**, in which the critical relative stereochemical relationships are established, is the intramolecular photocycloaddition of a

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